Culicoides imicola, biting midge species: a recent invader species in the Mediterranean basin ?

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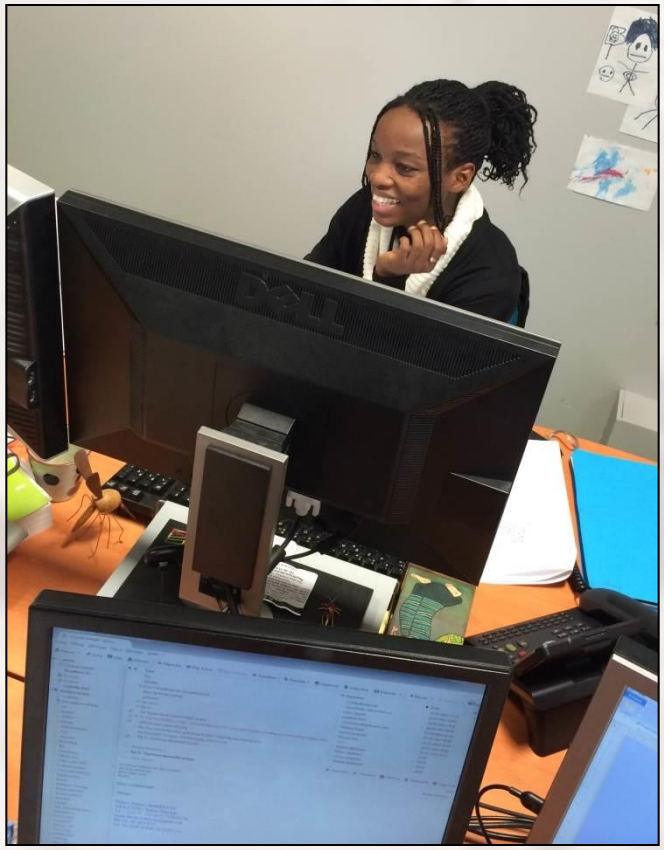
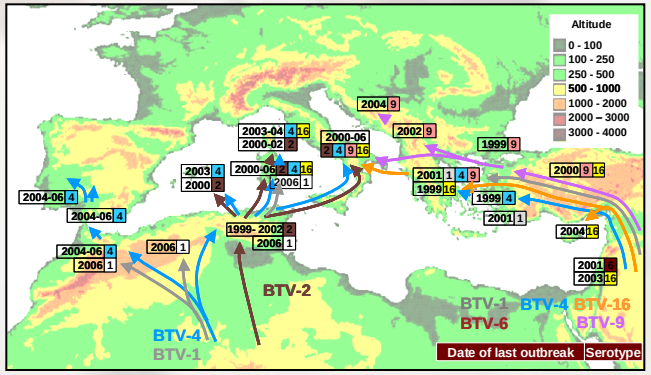


Culicoides imicola, biting midge vector species in expansion ?

- Small biting midge (Diptera : Ceratopogonidae), distributed in **Africa (native range)**, India, Middle and Near East

- Well known vector species of several economically important viruses of livestock : bluetongue virus (BTV), African horse sickness virus (AHSV), Epizootic hemorrhagic disease Virus (EHDV)

- The new circulation of BTV serotypes in the northern Mediterranean area from 2000 have revealed the presence of *C. imicola* populations in areas previously suspected as *C. imicola*-free



⇒ **Recent colonization *vs* entomological surveys bias ? Population structure and relationships between Mediterranean area and Africa ?**

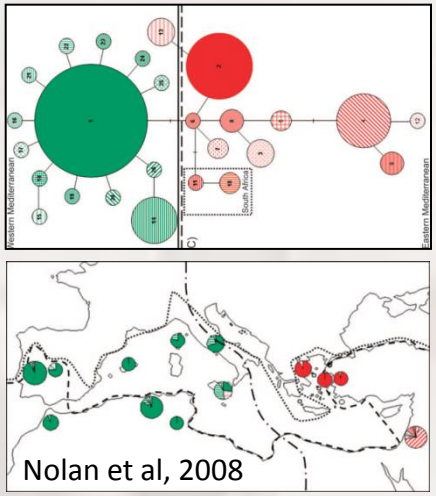
Phylogeographic data of *C. imicola* in the Mediterranean basin

- Several studies with single locus (COI) (Dallas et al 2003; Nolan et al 2008; Calvo et al 2009)
- Differentiation between East and West Mediterranean populations, north African populations related to West Mediterranean populations

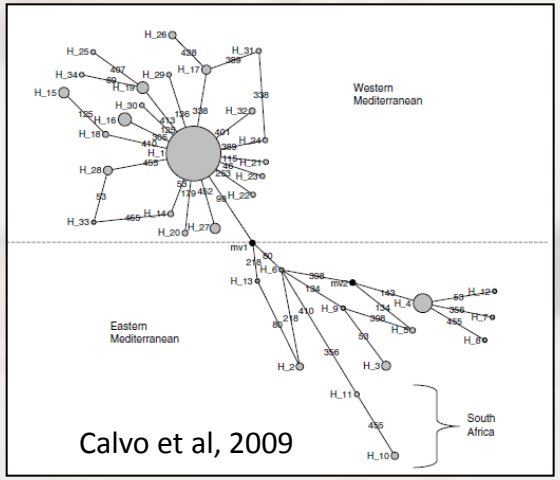
⇒ **Coherent with BTV serotypes circulation**

- Pattern of rapid and recent expansion populations in West Mediterranean area, not observed in East Mediterranean basin
- Recent development of microsatellite markers (Mardulyn et al 2013)

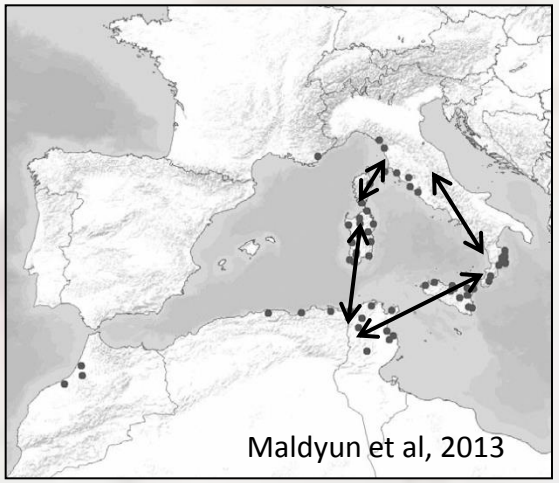
⇒ **Scenarios favored long time presence (30 years) of *C. imicola* in Italy and regular migration events**



Nolan et al, 2008



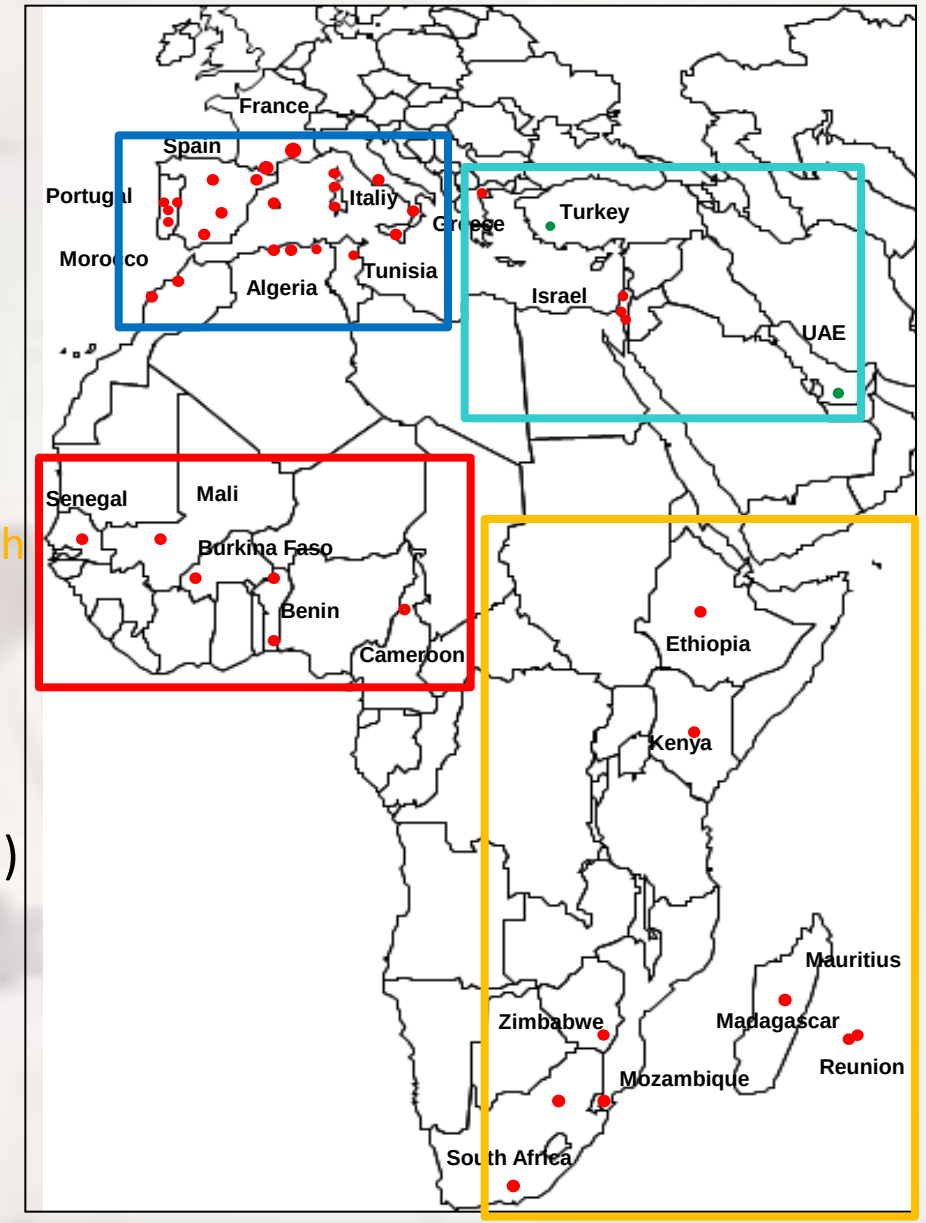
Calvo et al, 2009


Maldyun et al, 2013

Material and Methods : population set and markers used

- **55 populations for 24 territories**

West Mediterranean basin (WMB)
Portugal, Spain, France and island, Italy and islands, Morocco, Algeria, Tunisia
East Mediterranean basin (EMB)
Greece, Turkey, Israel
West Africa (WA)
Senegal, Mali, Burkina Faso, Benin, Cameroon
South-East Africa (SEA)
Ethiopia, Kenya, Zimbabwe, Mozambique, South Africa, Madagascar, Mauritius, Reunion island



- **3 molecular markers**

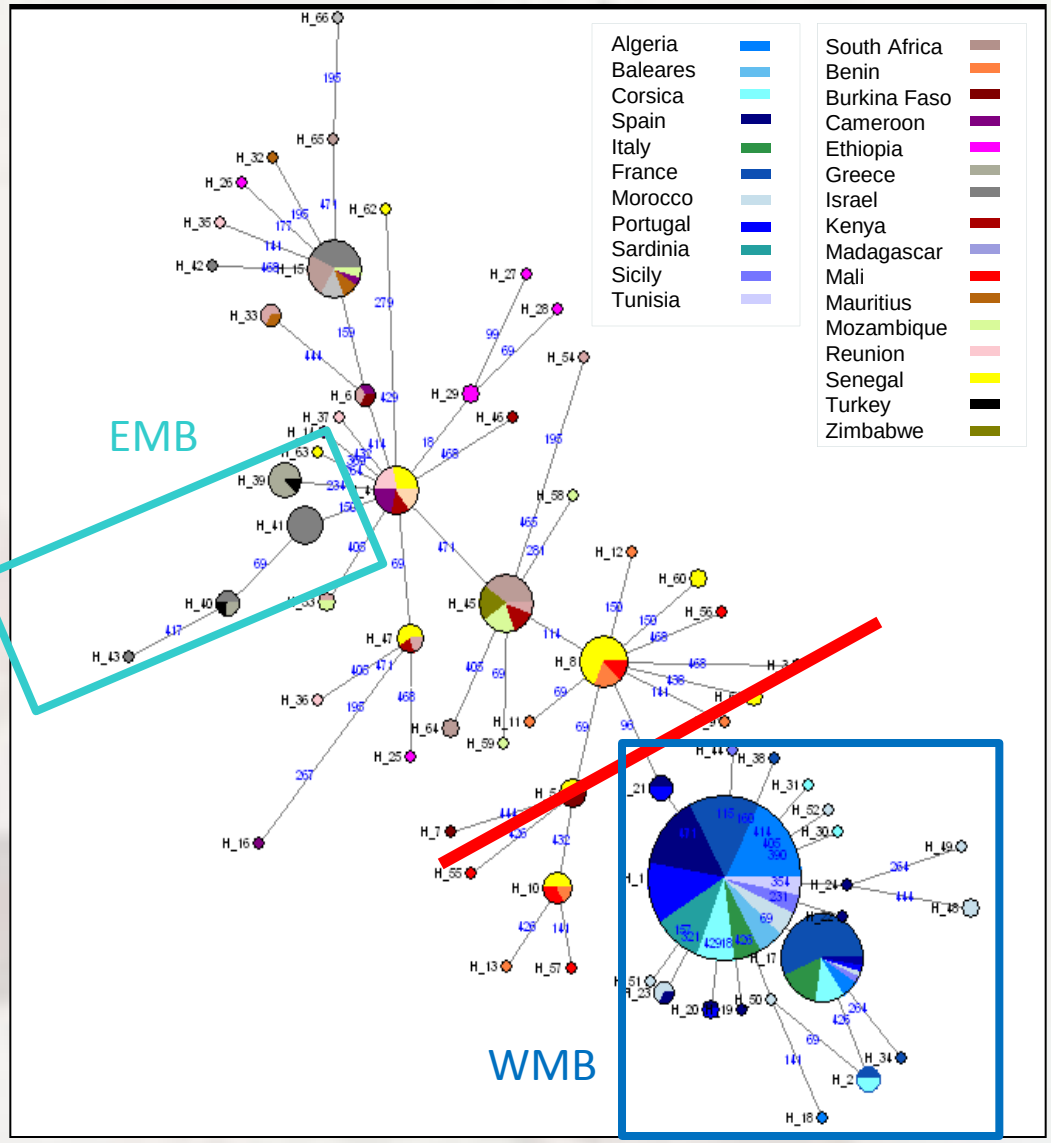
8 individuals/site
Cytochrome Oxidase subunit I (**COI**, 476bp)
Cytochrome B (**CytB**, 635bp)
Elongation factor alpha (**Efα**, 556bp)

- **9 microsatellites markers**

32 individuals/site

Results : marked genetic structure within the Mediterranean basin

- Differentiation of two groups : West Mediterranean basin (WMB) *vs* the others regions
- No shared haloptypes between the west (WMB) and east (EMB) Mediterranean basin
- Star-like network in the west Mediterranean basin (WMB) (signature of recent expansion ?)
- Same patterns with Cytb, although less informative



NJ network, COI polymorphism

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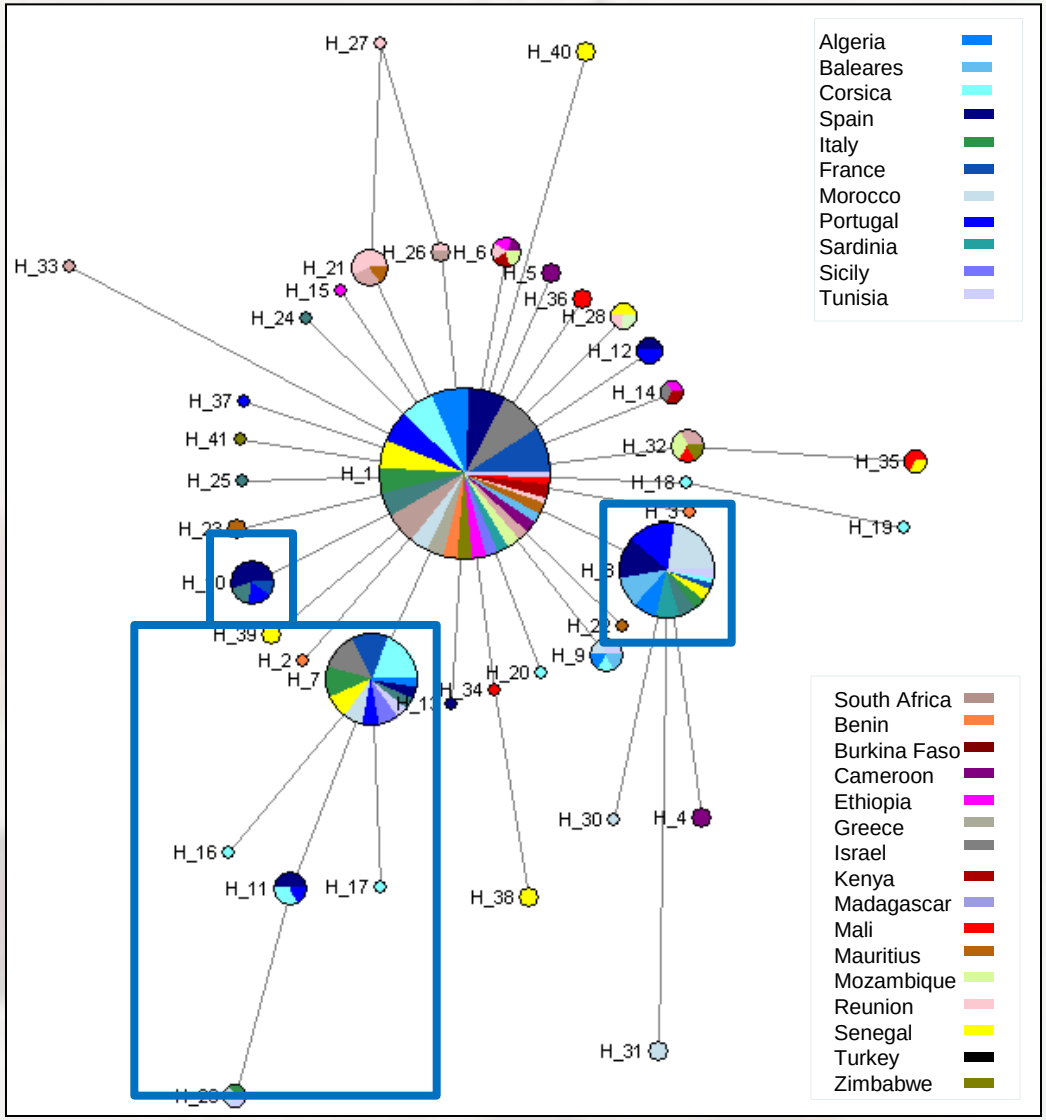
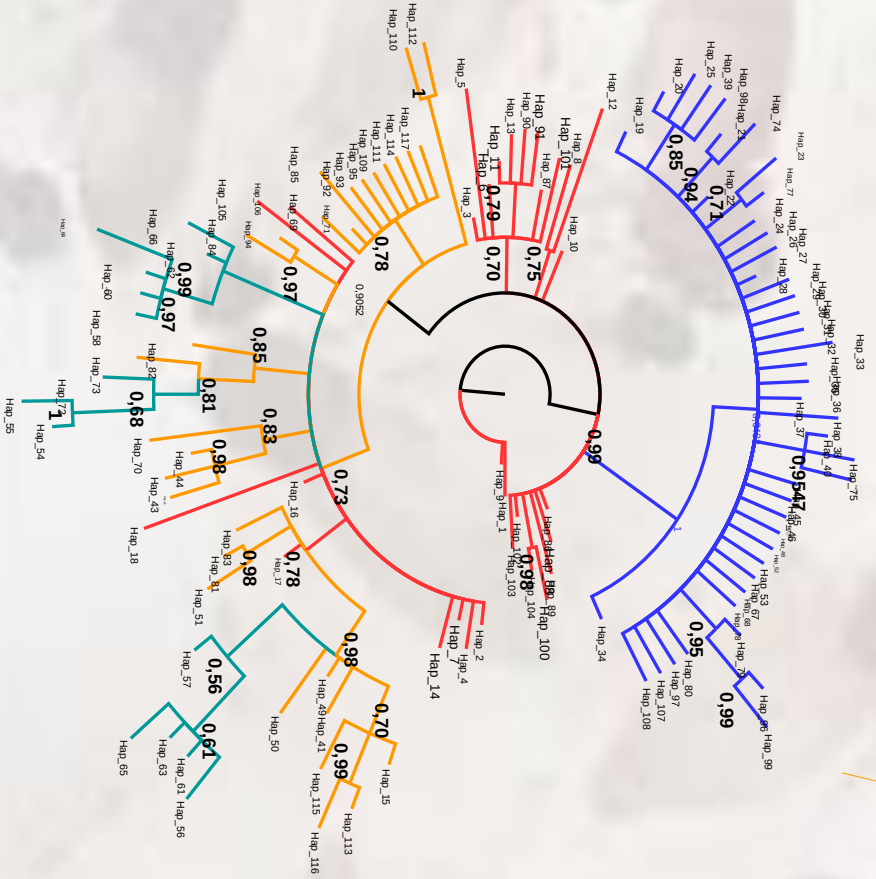
- Star-like network in the west mediterranean basin (WMB) (signature of recent expansion ?)
- same patterns with Cytb, although less unformative
- Slight differentiation in the WBM with the nuclear gene



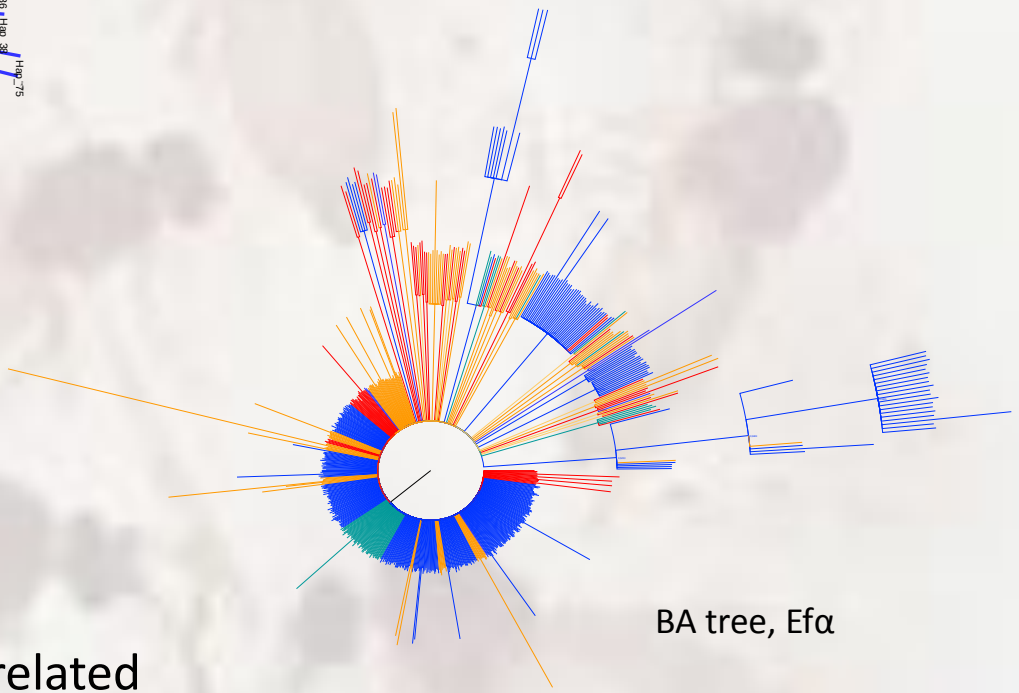
NJ network, Efα polymorphism

Results : one distinct clade in the West Mediterranean basin …



BA tree, COI+Cytb

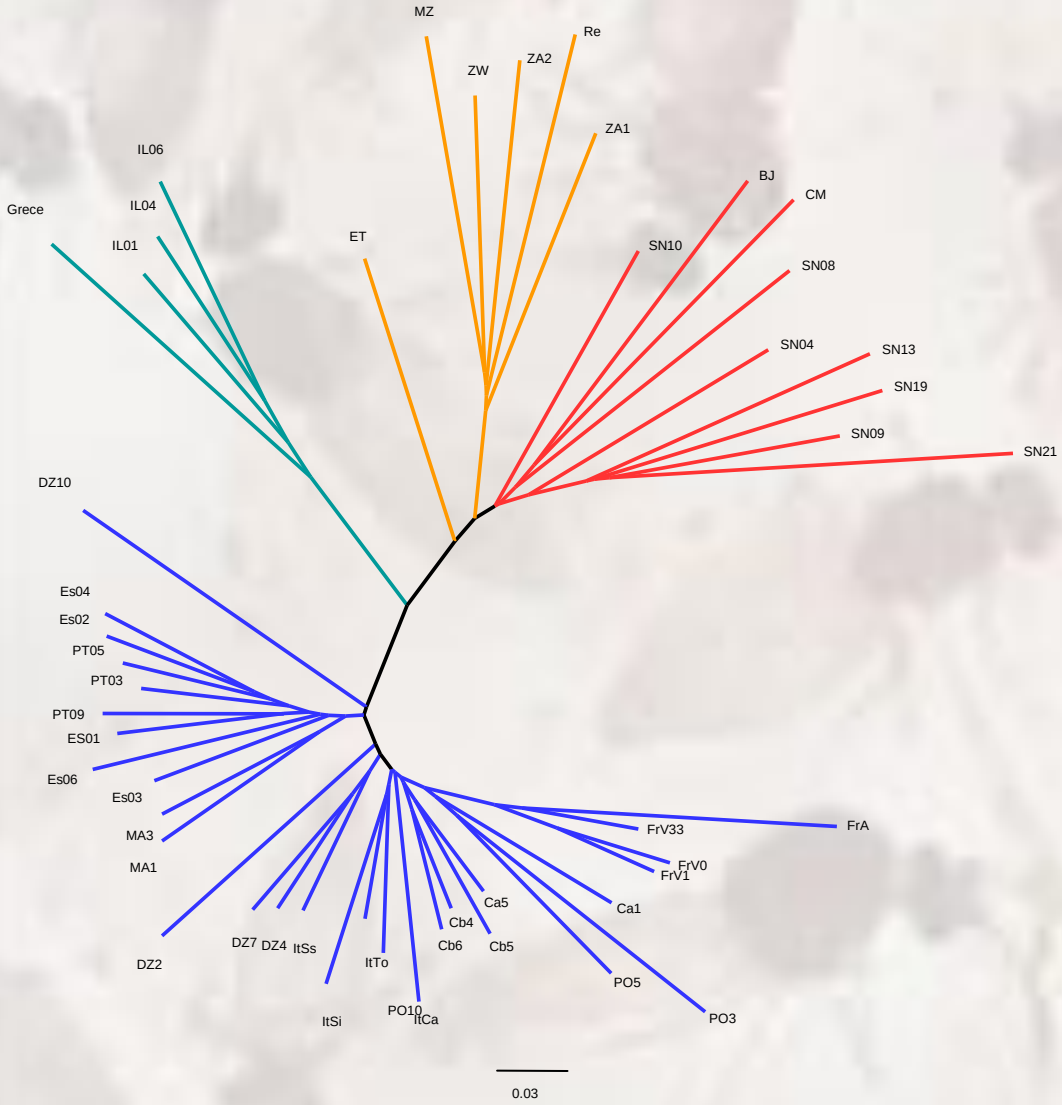
West Mediterranean basin
East Mediterranean basin
West Africa
South-East Africa



BA tree, Efα

- Populations from EMB genetically related to the populations from SEA or WA

Results : … and one distinct clade in West Africa !



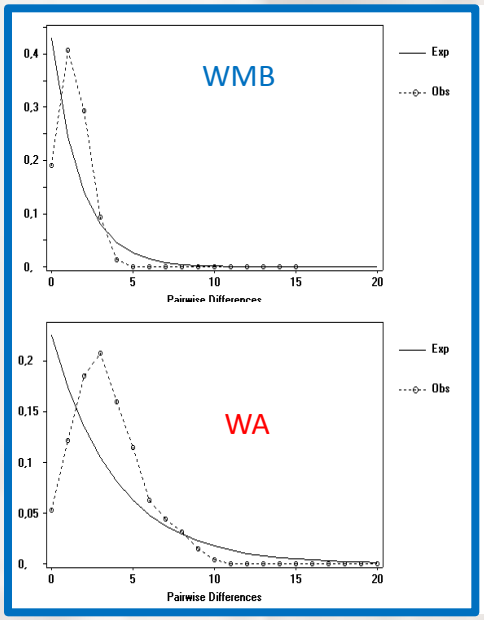
West Mediterranean basin
East Mediterranean basin
West Africa
South-East Africa

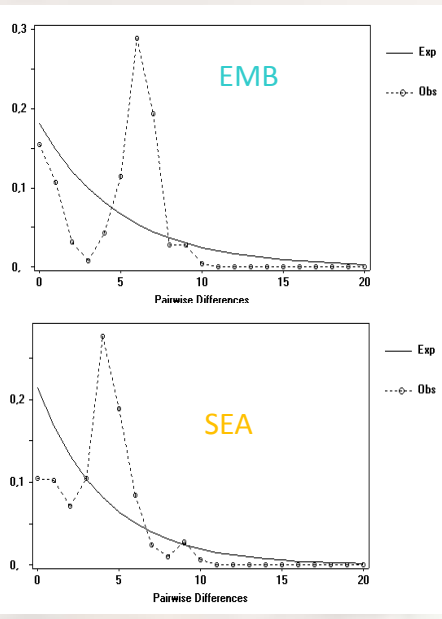
- Same structuration within the Mediterranean basin
- Differentiation in the sub-Saharan Africa with West Africa (WA) separated from SEA

Tree based on genetic distance of microsatellite polymorphism

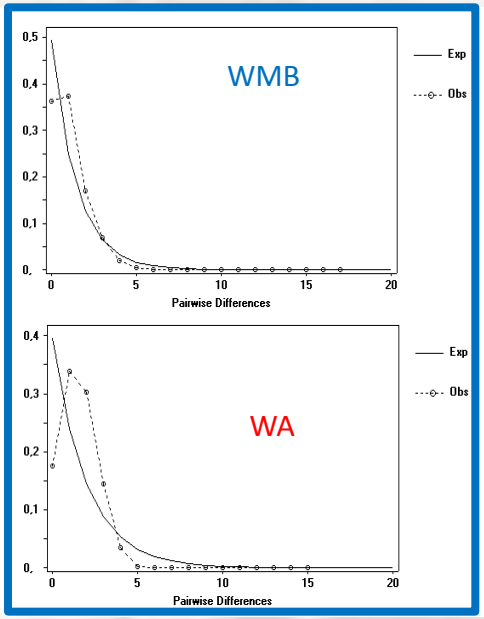
Results : clear signature of recent population expansion in 2 areas

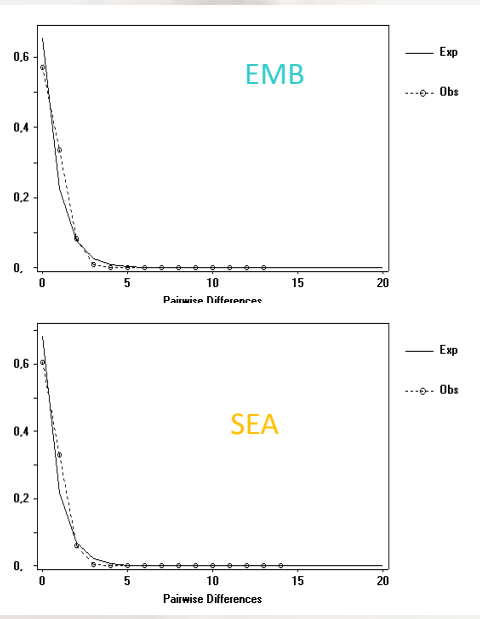
- Significant deviation from neutrality (COI+Cytb, Efα) for WA and WMB
- Demographic stability for SEA and EMB, while signature of population growth distribution for WMB and WA





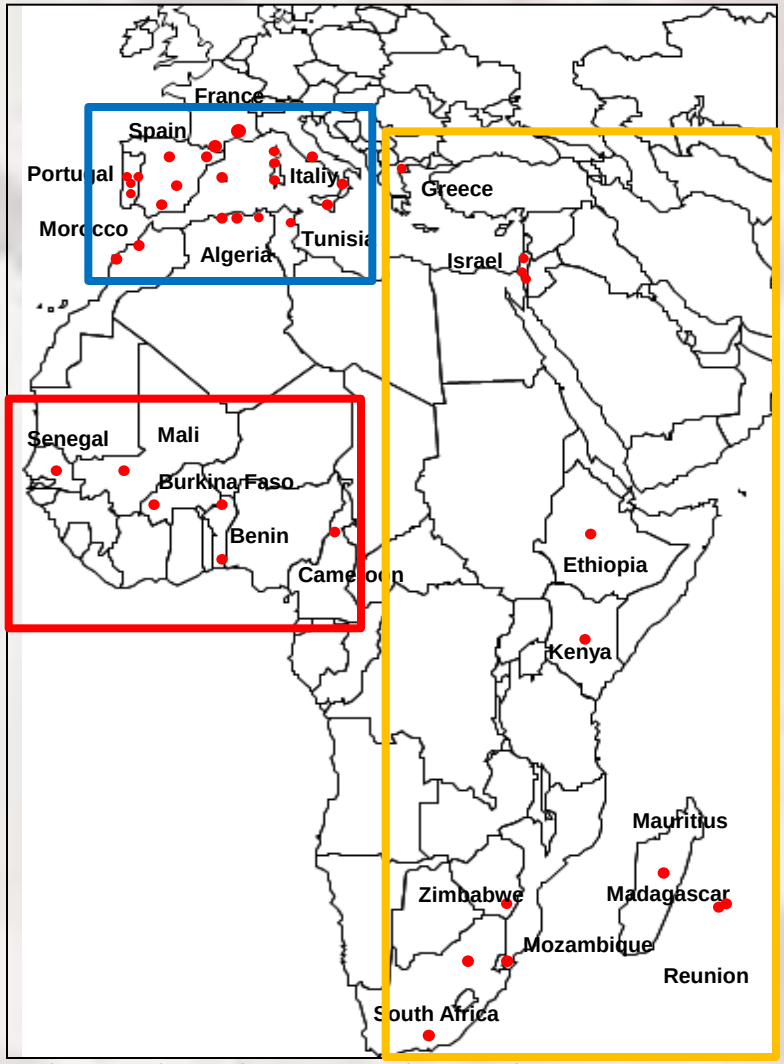
Mismatch analysis based on COI + Cytb





EFα

Results : consistent spatial genetic structure revealed



Clusters based on COI polymorphism

- Three separate clusters

	North Africa	Southern Europe	WA	SEA
Southern Europe	**0.051***	-		
WA	0.520**	0.603**	-	
SEA	0.614**	0.700**	0.236**	-
EMB	0.635**	0.715**	0.358**	0.167**

Fst per pair of populations

- **Low genetic differentiation between West Mediterranean populations**
- High genetic differentiation between West and East Mediterranean populations
- High genetic differentiation between West Mediterranean and African populations

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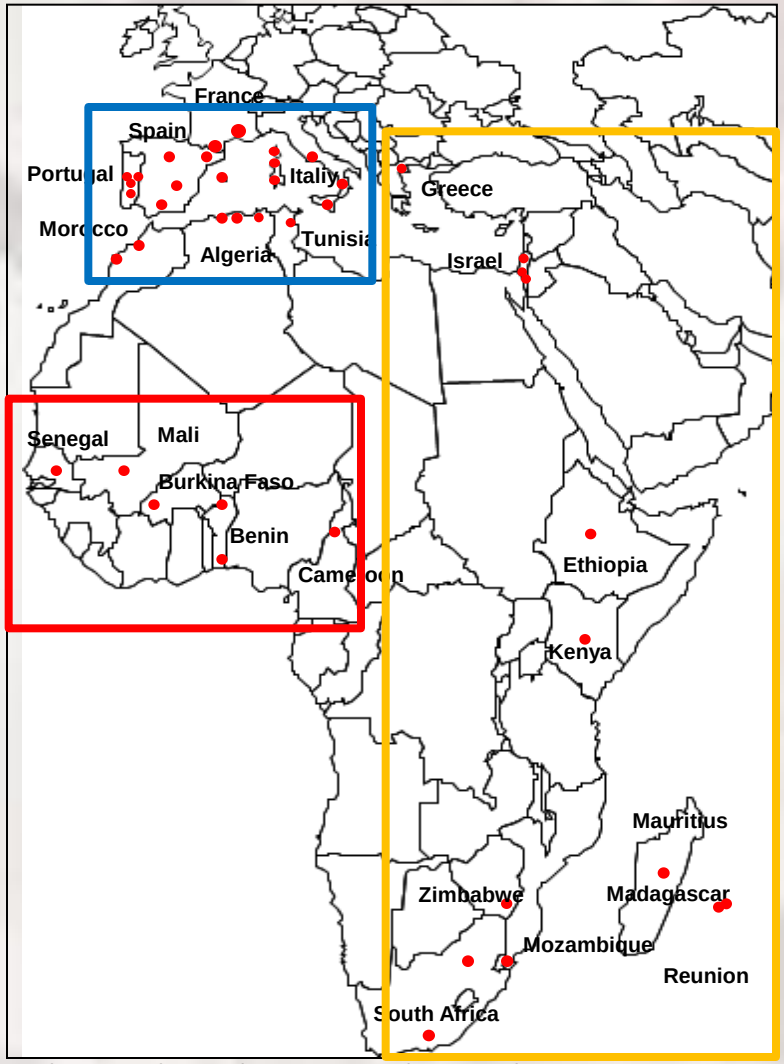

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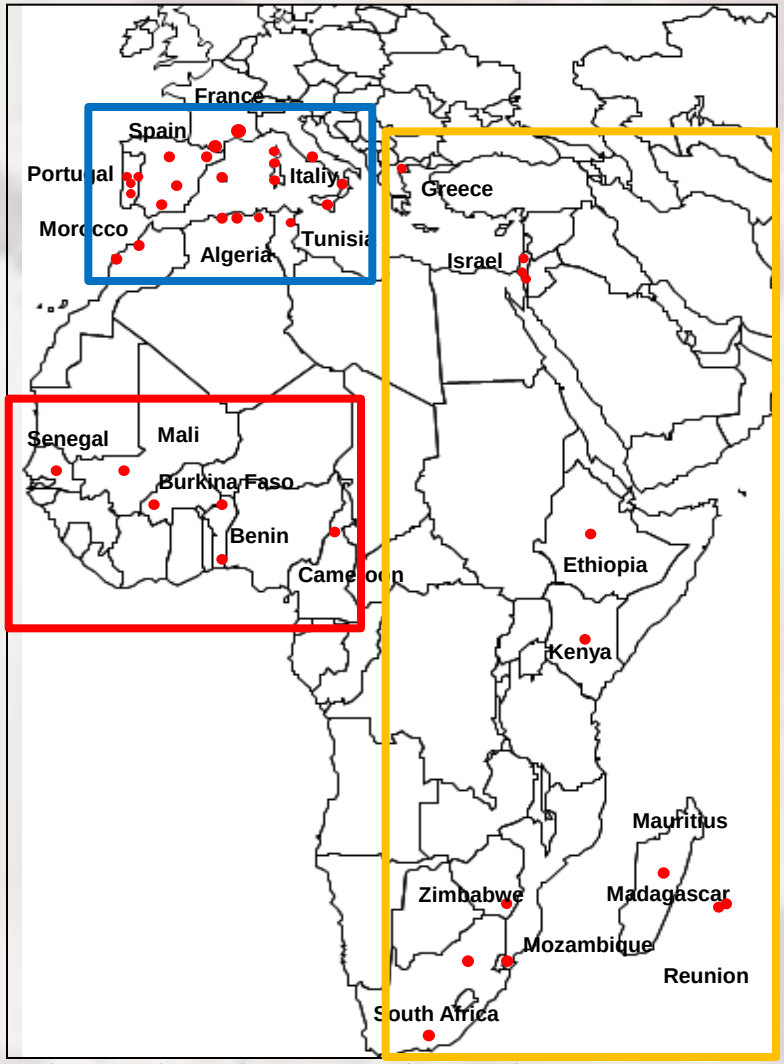

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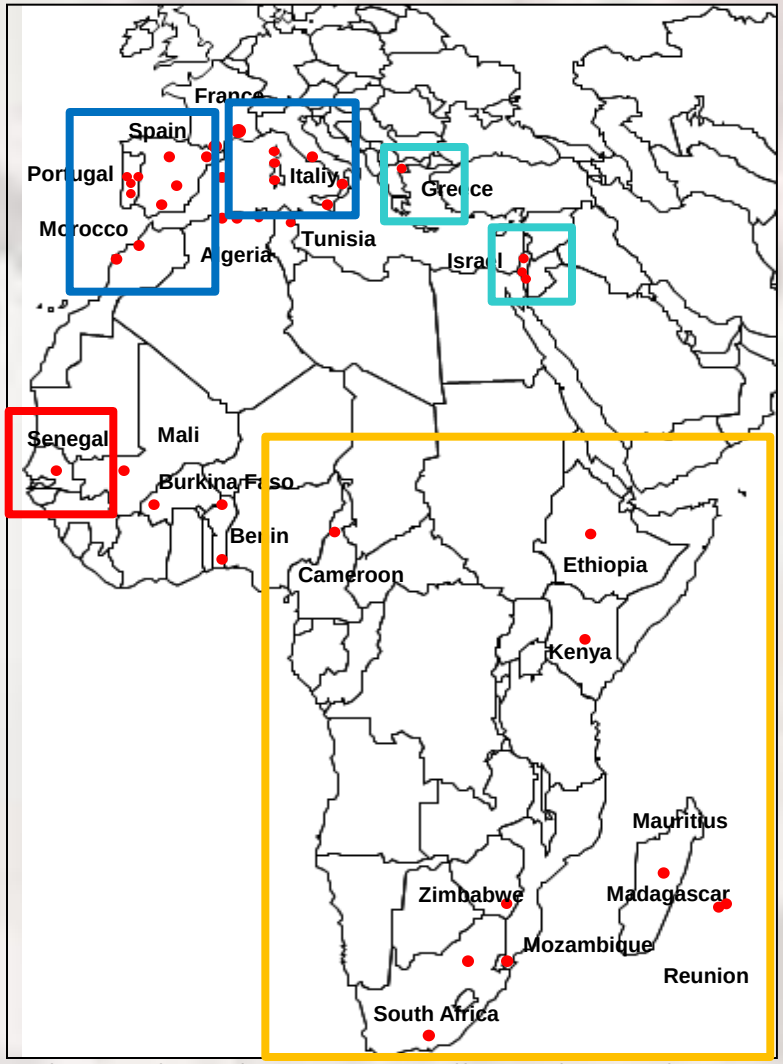

Clusters based on microsatellite polymorphism

- Six separate clusters
- **High genetic differentiation between West and East Mediterranean populations**
- High genetic differentiation between West Mediterranean and other sub-Saharan populations
- High differentiation between East Mediterranean and other sub-Saharan populations

Fst per pair of populations

	WMB	Central MB	Greece	Israel	SEA
Central MB	0.016**	-			
Greece	**0.147****	**0.113****	-		
Israel	**0.148****	**0.114****	0.078**	-	
SEA	0.097**	0.077**	0.137**	0.092**	-
Senegal	0.104**	0.078**	0.131**	0.128**	0.034**

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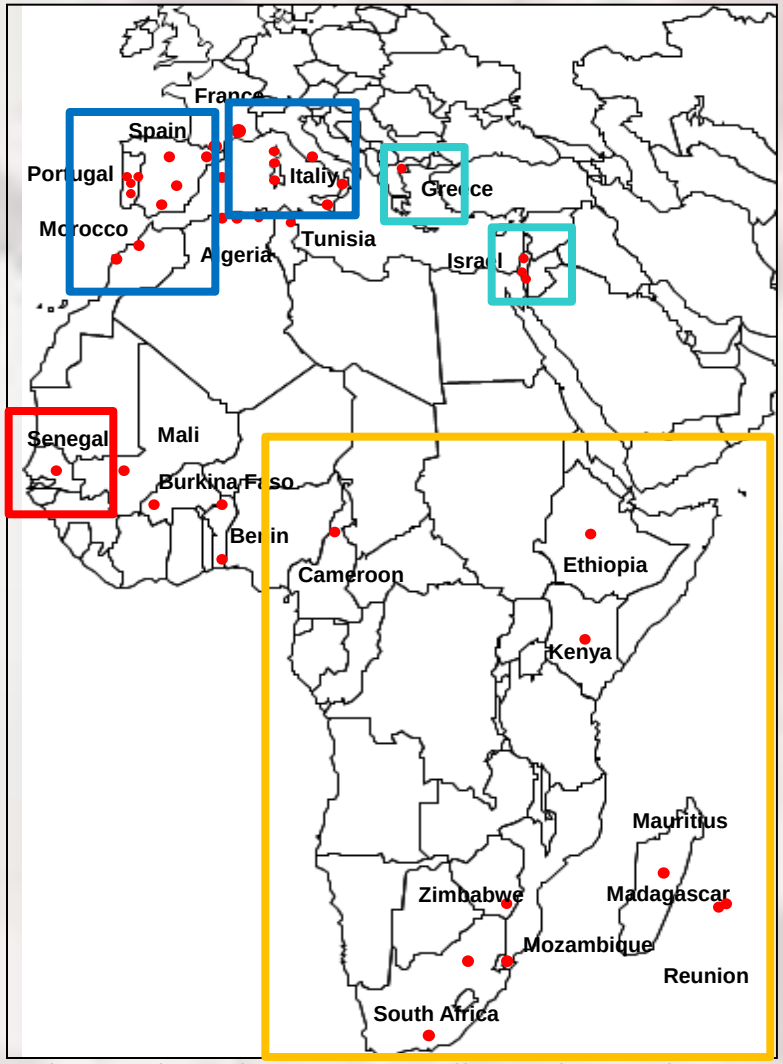

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Senegal	**0.104****	**0.078****	**0.131****	**0.128****	0.034**

Conclusions : *C. imicola*, invasive species ?

- High diversity in Africa and East Mediterranean basin + low diversity in the West Mediterranean basin
- Star-like shape network + signature of population growth/expansion in the West Mediterranean basin

⇒ **Historical range = sub-Saharan Africa**

⇒ **"Recent" demographic expansion of West Mediterranean populations, long time presence of *C. imicola* in the East Mediterranean basin**

- No shared haplotypes between Sub-Saharan Africa and West Mediterranean basin but West Mediterranean basin genetically closer to West Africa
- East Mediterranean basin genetically closer to South-East Africa

⇒ **Two corridors of expansion from Africa to Med : Atlantic coast and Nile valley**

- No shared haplotypes + high genetic differentiation between West and East Mediterranean basin

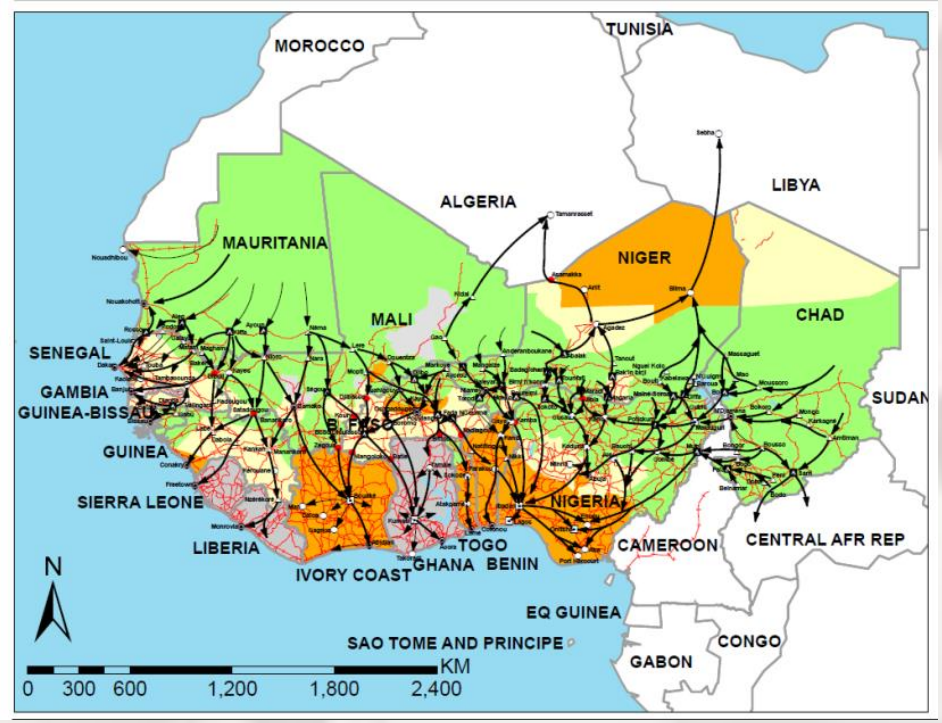
⇒ **Limited gene flow between East and West Mediterranean basin**

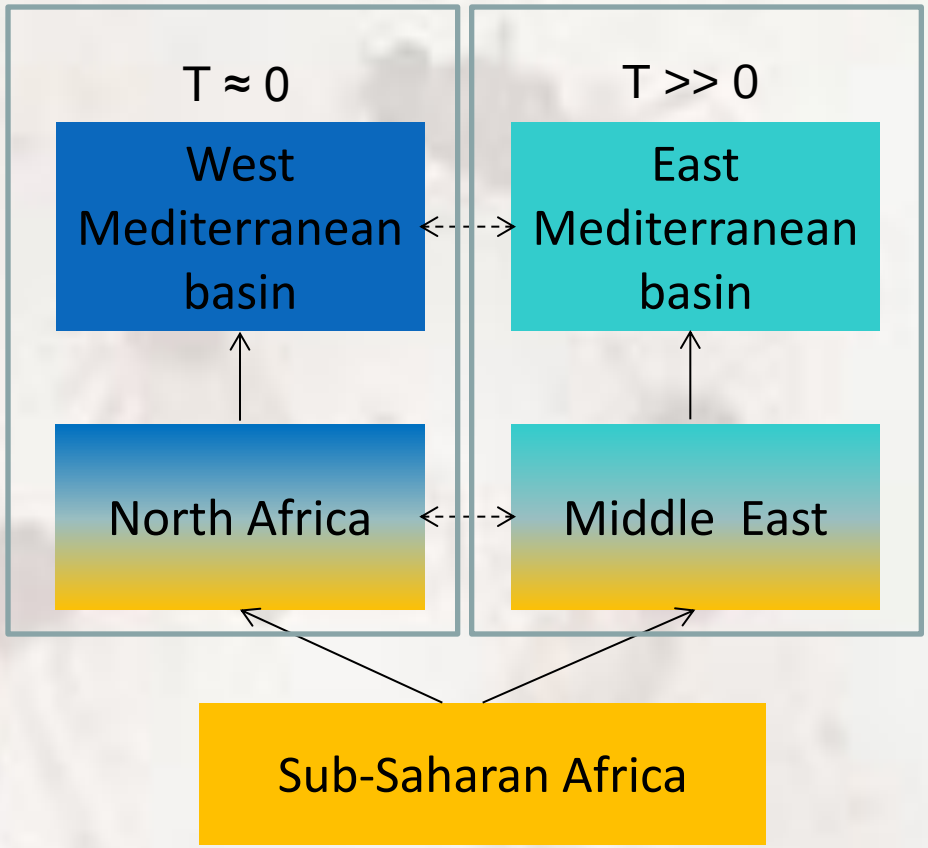
Conclusions : *C. imicola*, invader species in the West Med. area

- Support one source hypothesis

⇒ **Scenarios to be tested with ABC methods**

- In relation to past and current animal movements along the Atlantic coast corridor





Acknowledgements to the oversea Cirad colleagues and partners

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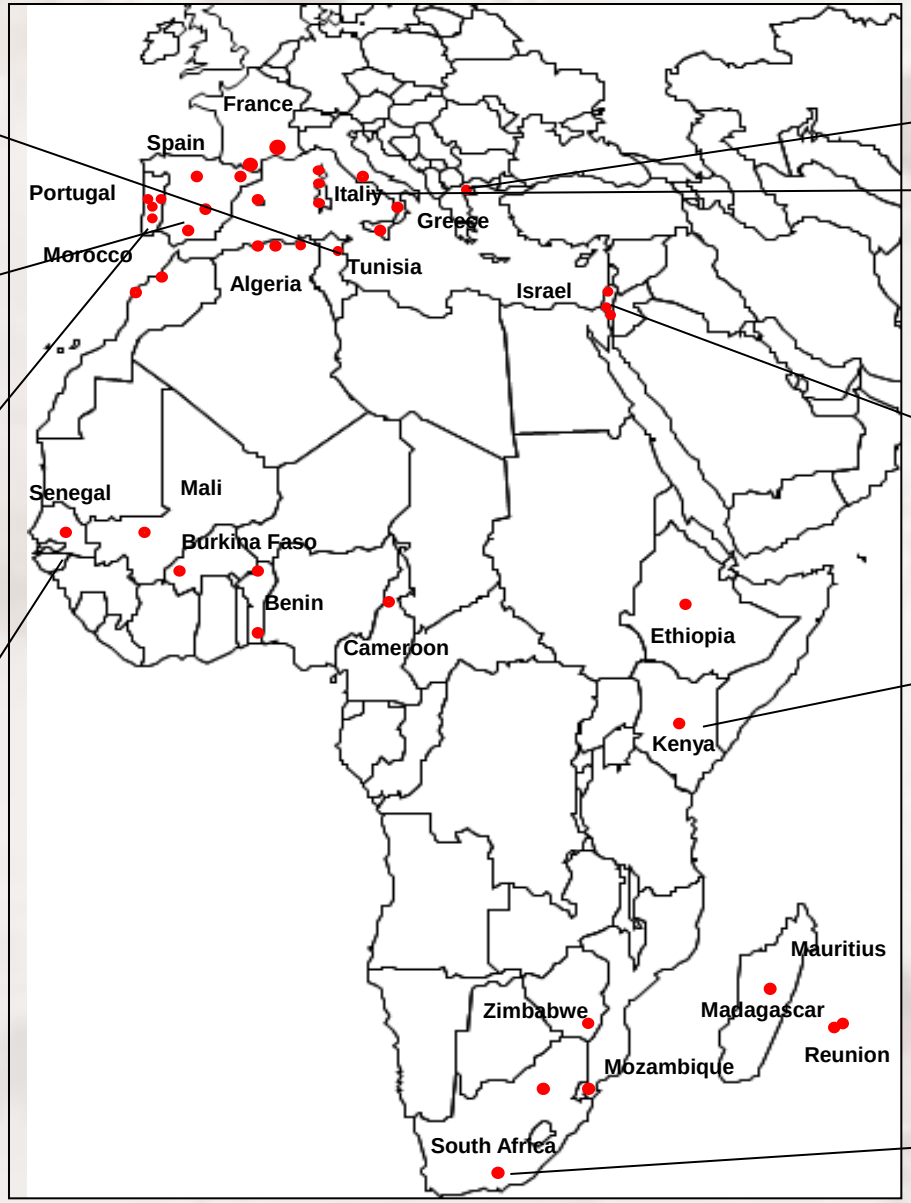

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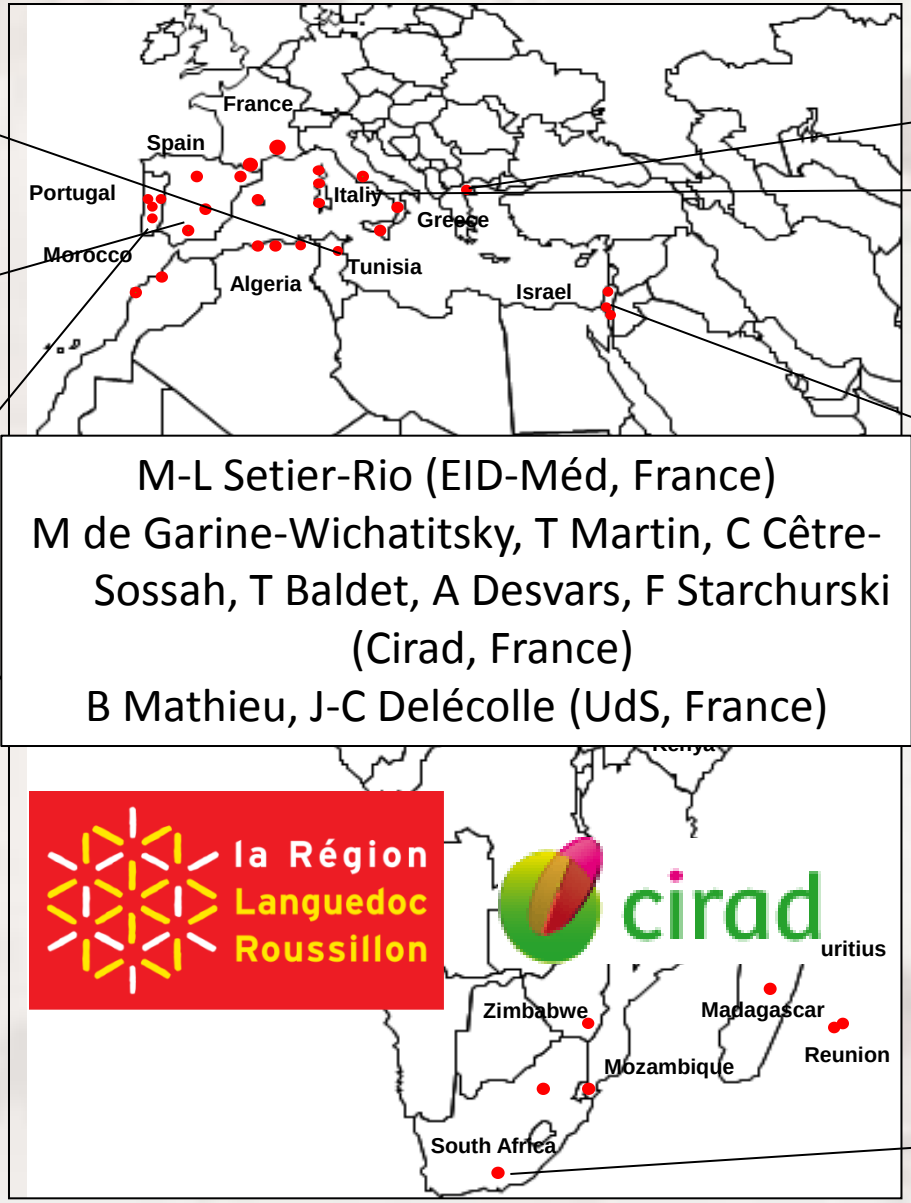

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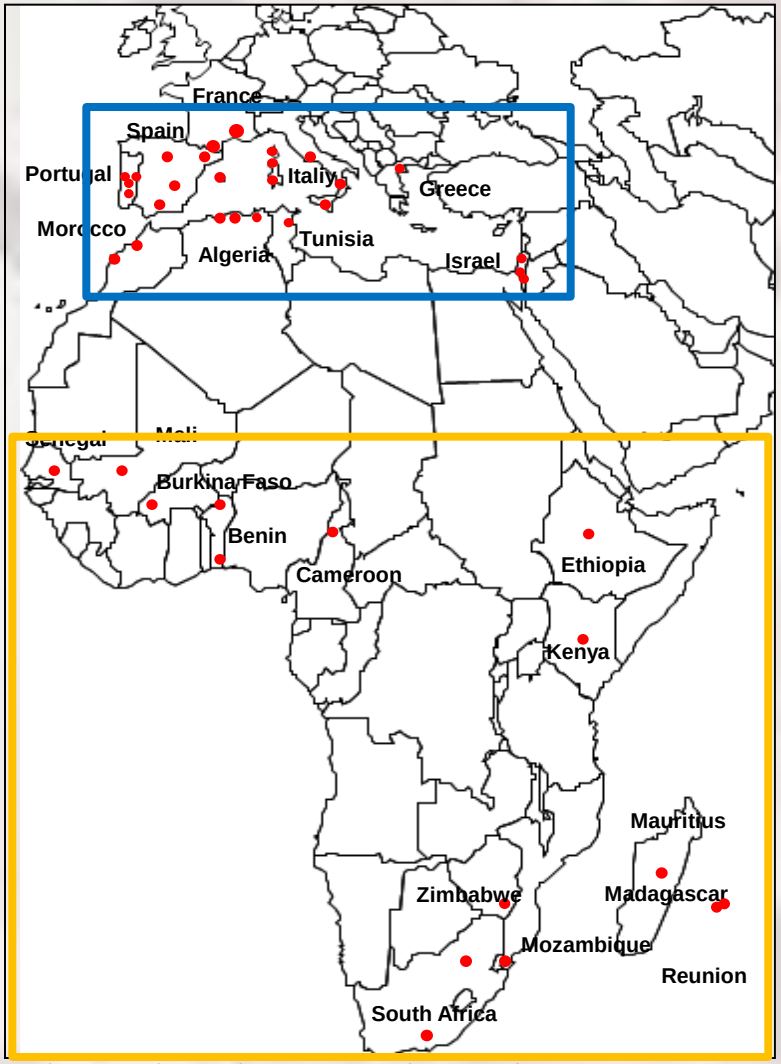
Many thanks for your attention

Results : high diversity in the native range

	COI + Cytb			Efα			Microsatellites	
Regions	**Haplotypes**	**Haplotype diversity**	**Nucleotide diversity**	**Haplotypes**	**Haplotype diversity**	**Nucleotide diversity**	**Mean nbr of alleles**	**Allelic richness**
WMB	**20**	**0.491**	**0.001**	26	0.637	0.0019	8.3	6.1
EMB	**41**	**0.721**	**0.004**	7	0.429	0.0009	7.2	6.7
WA + SEA	7	0.930	0.010	25	0.440	0.0011	**19.3**	**12.7**

- With mitochondrial markers, high haplotype diversity but low nucleotide diversity in WMB and EMB
- With microsatellite markers, diversity higher in Sub-Saharan Africa than in the Mediterranean basin

Results : consistent spatial genetic structure revealed



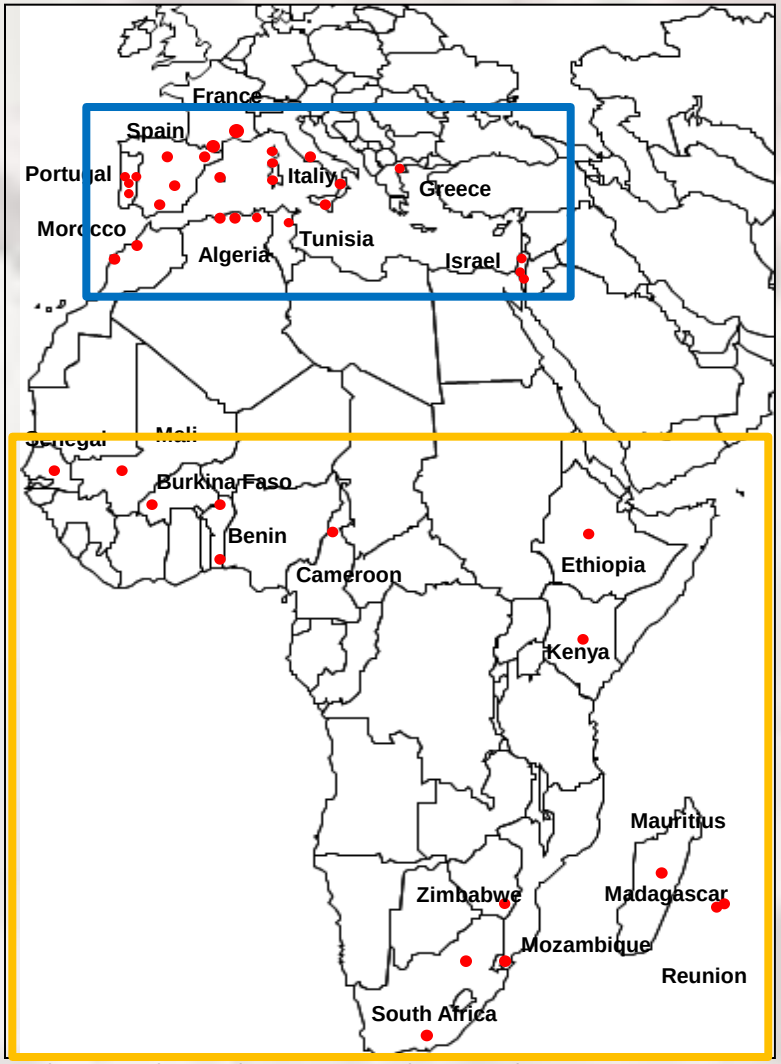
Clusters based on EFα polymorphism

- Two separate clusters
- **High genetic differentiation between West Mediterranean and African populations**
- Low genetic differentiation between East and West Mediterranean populations

	SEA	WA	EMB
WA	0.003**	-	
EMB	0.042**	0.045**	-
WMB	**0.712****	**0.659****	0.012

Fst per pair of populations

Results : consistent spatial genetic structure revealed



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